

# Bypassing antibiotic selection: positive screening of genetically modified cells with an antigen-dependent proliferation switch

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## ABSTRACT

**While antibiotic selection has been routinely used for the selection of genetically modified cells, administration of cytotoxic drugs often leads to deleterious effects not only to inert cells but also to transfected or transduced ones. In this study, we propose an Antigen-MEdiated Genetically modified cell Amplification (AMEGA) system employing antibody/receptor chimeras without antibiotic selection. Based on a rational design where the extracellular domains of dimeric erythropoietin receptor (EpoR) or gp130 were substituted with heterodimeric V<sub>H</sub>/V<sub>L</sub> regions of anti-hen egg lysozyme (HEL) antibody and EpoR D2 domains, the genes encoding the chimeras as well as a model transgene, enhanced green fluorescent protein (EGFP), were retrovirally infected into IL-3-dependent Ba/F3 cells followed by direct HEL selection in the absence of IL-3. After a single round of selection, EGFP-positive cells were selectively amplified, resulting in a population of almost 100% positive cells. The AMEGA without antibiotic selection will not harm normal cells, which will be especially useful for increasing the efficacy for stem cell-based gene therapy.**

## INTRODUCTION

Selection of genetically modified cells is a critical step to engineer the cells with desired properties. Antibiotic selection has been commonly used, which is based on the rescue of the transfectant in the presence of a cytotoxic drug and the selective deprivation of the non-transduced cells. However, the selection conditions such as initial cell density and drug concentration should be carefully determined since inappropriate conditions often lead to death of the transfectant or

survival of the parental cells (1). Furthermore, the growth rate is decreased even in the optimal concentration due to the cytotoxic drug (2,3). Such a selection procedure has some limitations for cells in which transduction efficiency is low and/or growth induction is difficult (1,4–6).

To overcome these problems, we tried to create an artificial receptor that transduces a growth signal in response to a non-toxic substance. Co-expression of the artificial receptor and the gene of interest would result in a 'growth advantage' only for genetically modified cells by simply adding the non-toxic substance in the culture medium. We focused on an antigen-antibody system that has an infinite number of combinations with high specificity. In our previous study, the Fv region of anti-hen egg lysozyme (HEL) antibody HyHEL-10 was chosen as a model, since its V<sub>H</sub>-V<sub>L</sub> association is greatly enhanced with addition of HEL (7), which is expected to be suitable for mimicry of ligand-induced dimerization of cytokine receptors in their activation (8,9). Thus, two chimeric erythropoietin receptors whose extracellular domain was replaced with either the V<sub>H</sub> or V<sub>L</sub> region of anti-hen egg lysozyme (HEL) antibody HyHEL-10 (HE or LE, respectively) were expressed in interleukin-3 (IL-3)-dependent hematopoietic cell lines, resulting in HEL-dependent cell growth without IL-3 (10). This result demonstrates the utility of our antibody/receptor chimera for functional mimicry of cytokine receptors. In the following experiment, the cytoplasmic domains of HE and LE chimeric receptors were replaced with that of gp130 to create Hg and Lg chimeric receptors, respectively. Co-expression of Hg and Lg or Hg and LE resulted in HEL-dependent cell growth in factor-dependent cell lines (11,12). Furthermore, when the enhanced green fluorescent protein (EGFP) was employed as a model transgene and placed downstream of the LE gene and the internal ribosomal entry site (IRES), HEL induced specific amplification of the cells with high expression level of EGFP (13). However, in all these studies, we used electroporation to transfect plasmids into the cells, which is not an ideal method for many applications due to relatively low transfection

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efficiency. In addition, in order to enrich the proportion of transfectants, antibiotic selection was always conducted prior to HEL selection, and the possibility of direct HEL selection was not pursued.

In this study, we employed a retroviral vector to attain higher transduction efficiency in a variety of cell types to expand the current approach. Whether or not the genetically modified IL-3-dependent pro-B cells expressing the antibody/receptor chimera could be directly amplified by HEL selection immediately after retroviral infection was examined.

## MATERIALS AND METHODS

### Vector construction

The ecotropic retroviral vector pMX, and its derivatives pMX-GFP and pMXs-neo (14), were kindly provided by Dr T. Kitamura (Institute of Medical Science, University of Tokyo). The construction of the HE chain gene encoding HyHEL-10 V<sub>H</sub>, a GSG linker, extracellular D2 and transmembrane/cytoplasmic domains of EpoR, and the LE chain gene encoding HyHEL-10 V<sub>L</sub> instead of V<sub>H</sub> in the HE chain, was described previously (10). Construction of the Hg and Lg chain genes encoding the transmembrane and cytoplasmic domains of gp130 instead of those for HE and LE chains, respectively, was as described (11). First, the 'amplifier' vectors were constructed. The Hg and Lg genes were digested from pME-Hg and pMEZ-Lg (11) with *EcoRI*, and ligated into *EcoRI*-digested pMX to obtain pMX-Hg and pMX-Lg, respectively. pME-HE (11) was digested with *NcoI* and *XbaI* to obtain the HE chain fragment, and inserted into *NcoI* and *XbaI*-digested pBSN, which is a variant of pBluescriptII SK(-) (Stratagene, La Jolla, CA) having a *NcoI* site between the *SpeI* and *XbaI* sites, in order to make a *NotI* site downstream of the HE gene, resulting in pBSN-HE. The HE gene fragment was digested from pBSN-HE with *NcoI* and *NotI*, and ligated into *NcoI* and *NotI*-digested pMX-Hg to produce pMX-HE. pMEZ-LE was digested with *NcoI* and *SacII* to obtain the V<sub>L</sub> fragment, and ligated into *NcoI* and *SacII*-digested pMX-HE to obtain pMX-LE. The 'courier' vectors were constructed as follows. pMEZ-LEIGFP (13) was digested with *XbaI* to obtain the IRES-EGFP fragment, and inserted into a *XbaI* site of pBluescriptII SK(-) to produce pBS-IGFP. The IRES-EGFP fragment from pBS-IGFP was digested with *NotI*, and inserted into a *NotI* site of pMX-Hg and pMX-Lg to get pMX-HgIGFP and pMX-LgIGFP, which encode the IRES-EGFP cassette downstream of Hg and Lg, respectively. The LEIGFP fragment was digested from pMEZ-LEIGFP (13) with *EcoRI* and *NotI*, and inserted into *EcoRI* and *NotI*-digested pMX to create pMX-LEIGFP. To make a drug-resistant control vector, pMX-LgIGFP was digested with *EcoRI* and blunted with T4 DNA polymerase, followed by *XhoI* digestion to remove the Lg fragment. pMXs-neo was digested with *BglII* and blunted, followed by *SalI* digestion to obtain the neomycin-resistance gene fragment. The fragment was inserted into the digested pMX-LgIGFP to obtain pMX-neoIGFP.

### Cell culture

A murine IL-3-dependent pro-B cell line, Ba/F3 (15), was cultured in RPMI 1640 medium (Nissui Pharmaceutical,

Tokyo, Japan) supplemented with 10% FBS (Iwaki, Tokyo, Japan) and 2 ng/ml murine IL-3 (Genzyme/Techne, Cambridge, MA). A retroviral packaging cell line, Plat-E (16), was cultured in Dulbecco's modified Eagle's medium (DMEM) (Nissui Pharmaceutical) supplemented with 10% FBS, 1 µg/ml puromycin (Sigma, St Louis, MO) and 10 µg/ml blasticidin (Kaken Pharmaceutical, Tokyo, Japan).

### Retroviral infection and selection of the transfectants

Plat-E cells were inoculated into a 60 mm diameter dish at  $5 \times 10^5$  cells/ml in 4 ml DMEM, and cultured for 20 h. An aliquot of 9 µl Fugene6 (Roche Diagnostics, Basel, Switzerland) was diluted with 100 µl of serum-free F12 medium (Gibco BRL, Rockville, MD) and added to 3 µg of the retroviral vector solubilized in 6 µl of sterile water. After 15 min incubation at room temperature, the vector/Fugene6 mixture was added to the Plat-E cells. After 24 h incubation, the culture medium was refreshed with 4 ml DMEM, followed by an additional 24 h incubation. After the supernatant was centrifuged at 1000 g for 5 min at 20°C, Ba/F3 cells ( $10^5$  cells) were infected with 500 µl of the viral supernatant in the presence of 10 µg/ml polybrene (Sigma) and 4 ng/ml IL-3 in 24-well plates. In the case of co-infection, 500 µl each of the viral supernatants was mixed and used to infect Ba/F3 cells. After 5 h incubation, the same volume of RPMI 1640 as the viral supernatant was added to reduce the toxicity of polybrene. The approximate multiplicity of infection (MOI) ranged from 1.0 to 4.2%, as estimated from the flow cytometric analysis on day 3 or 4 after infection. On day 6 or 7 after infection, the cells were washed with PBS once, and inoculated into 24-well plates at  $2 \times 10^5$  cells/ml. Selection was performed in the medium containing no factor, 1 µg/ml HEL (Seikagaku Corporation, Tokyo, Japan) or 2 ng/ml IL-3.

For the cloning experiment, cells before selection were washed with PBS once, and inoculated into 96-well plates at  $5 \times 10^3$  cells/ml ( $10^3$  cells/well) with 1 µg/ml HEL. Parental Ba/F3 cells were washed with PBS three times and used to supplement each well at  $10^5$  cells/ml as feeder cells to effectively rescue limiting-diluted transfectant cells in their early growth phase. For comparison, retrovirally packaged pMX-neoIGFP was infected into Ba/F3 cells (MOI ~35%), followed by inoculation into 96-well plates at 5 cells/ml (1 cell/well) with 2 ng/ml IL-3 and 800 µg/ml G418 (Calbiochem, Darmstadt, Germany). Washed Ba/F3 cells ( $10^5$  cells/ml) were used to supplement each well as feeders.

### Western blotting

The cells ( $10^6$  cells) were washed with PBS, lysed with 100 µl of lysis buffer [20 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl<sub>2</sub>, 1 mM EGTA, 10 µg/ml aprotinin, 10 µg/ml leupeptin] and incubated on ice for 10 min. After centrifugation at 16 000 g for 5 min, the supernatant was mixed with Laemmli's sample buffer and boiled. The lysate was resolved by SDS-PAGE and transferred to a nitrocellulose membrane (Millipore, Bedford, MA). After the membrane was blocked with 5% skimmed milk, the blot was probed with 160 ng/ml of rabbit anti-mouse EpoR antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or 160 ng/ml of anti-mouse gp130 antibody (Santa Cruz Biotechnology) followed by 560 ng/ml of HRP-conjugated anti-rabbit IgG (Biosource, Camarillo, CA), and detection was

performed using the ECL system (Amersham-Pharmacia, Little Chalfont, UK).

### Cell proliferation assay

The HEL-selected cells were washed three times with PBS and seeded in 24-well plates containing various concentrations of HEL. The initial cell concentration was adjusted to  $10^4$  cells/ml. Cell number and viability were determined using a hemocytometer and the trypan blue exclusion assay.

### Flow cytometry

Cells were washed with PBS and resuspended in Isoflow (Beckman Coulter, Fullerton, CA). Green fluorescence intensity was measured using an EPICS-ALTRA flow cytometer (Beckman Coulter) at 488 nm excitation and fluorescence detection at  $525 \pm 20$  nm.

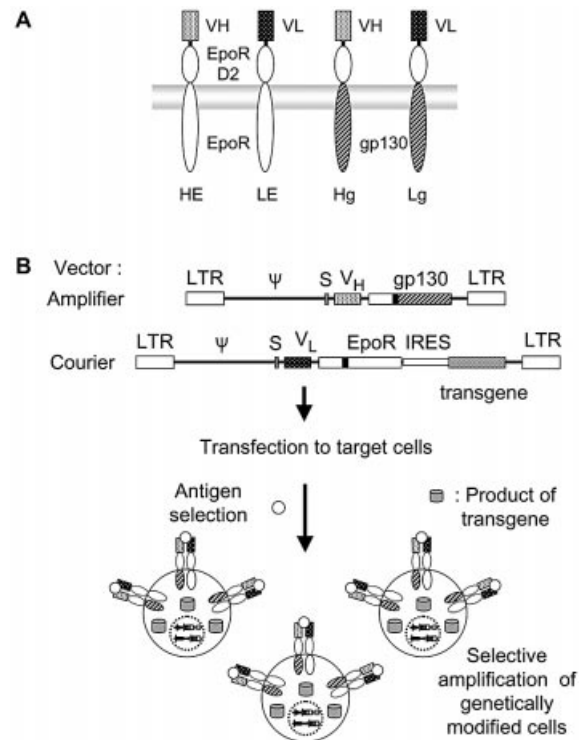
## RESULTS

### Direct HEL selection amplified the transfectants expressing chimeric receptors

We first designed a selection scheme of the direct antigen-mediated selection without the antibiotic selection step (Fig. 1). An 'amplifier' vector encodes a chimeric receptor gene, while a 'courier' vector encodes the other partner of the functional chimeric receptor pair and an IRES-transgene cassette. The IRES sequence was used to couple a growth signal derived from chimeric receptors with transgene expression, since a correlation was observed between the expression levels of the two cistrons placed upstream and downstream of the IRES sequence (17,18). Therefore, transgene-expressing cells are expected to preferentially proliferate in the antigen-containing medium.

In this study, the EGFP gene was used as a model transgene, and an IRES-EGFP cassette was designated as IGFP. Four types of 'amplifier' vectors (pMX-HE, pMX-LE, pMX-Hg and pMX-Lg) and three types of 'courier' vectors (pMX-LEIGFP, pMX-HgIGFP and pMX-LgIGFP) were constructed. IL-3-dependent Ba/F3 cells were infected with either one of the courier vectors or with one of the amplifier-courier combinations, resulting in Ba/LEIGFP, Ba/HgIGFP, Ba/LgIGFP, Ba/HgIGFP+Lg, Ba/HE+LgIGFP, Ba/Hg+LEIGFP and Ba/HgIGFP+LE cells. On day 6 or 7 after infection, the cells were washed with PBS once, and selected in the medium containing no factors, 1  $\mu$ g/ml HEL or 2 ng/ml IL-3. Ba/LEIGFP, Ba/HgIGFP and Ba/LgIGFP cells died immediately in the selection medium without IL-3 (data not shown), indicating that these courier vectors have no activity for selective amplification of the transfectants on their own. On the other hand, all transfectants with amplifier-courier combinations gave proliferating cells with HEL selection. Ba/HgIGFP+Lg and Ba/HE+LgIGFP cells proliferate even without any factors, indicating the background growth-stimulating activity of the Hg+Lg and HE+Lg combinations.

To investigate whether cell proliferation in the selection medium with HEL or no factors was induced by the expressed chimeric receptors, cell lysates before and after the selection were prepared and the amounts of expressed chimeric receptors was compared by western blotting using anti-EpoR or anti-gp130 antibodies (Fig. 2). In all amplifier-courier combinations, IL-3-selected cells expressed ~2-fold less

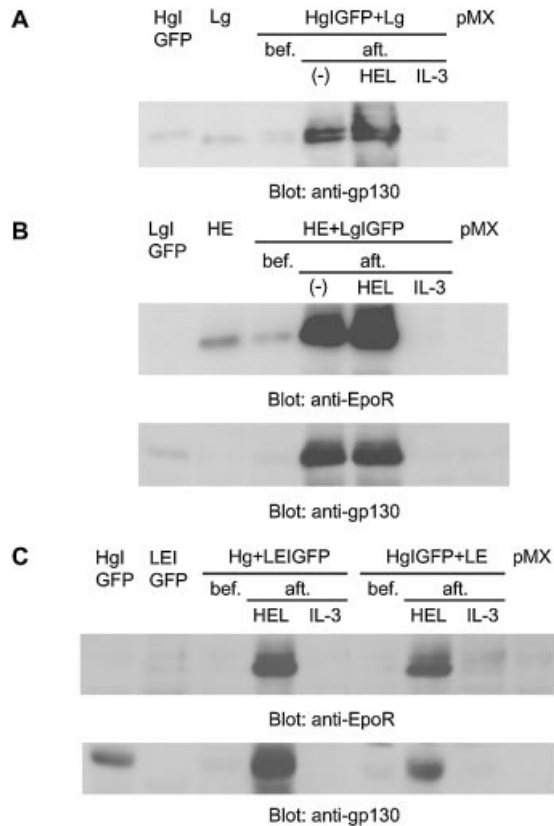


**Figure 1.** Scheme of the AMEGA system. (A) Chimeric receptor constructs used in this study. The extracellular N-terminal half of EpoR was replaced with either the V<sub>H</sub> or V<sub>L</sub> region of HyHEL-10 with a Gly-Ser-Gly tripeptide linker between V<sub>H</sub>/V<sub>L</sub> and Val118 of the EpoR D2 domain. The transmembrane and intracellular domains could be easily replaced at the *EcoRV* site inserted at the C-terminus of the EpoR D2 domain. (B) Schematic diagram of the selection procedure. Chimeric receptor combination of Hg and LE is depicted in the scheme as a representative. Retroviral vectors with long-terminal repeats (LTRs) and a packaging signal ( $\psi$ ) are used for the efficient transcription of the Hg and LE-IRES-transgene. An immunoglobulin heavy-chain secretion signal sequence (S) is located upstream of the chimeric receptor genes to enable their cell surface expression.

amounts of chimeric receptors than the cells before selection. On the other hand, HEL-selected cells expressed considerably higher amounts of chimeric receptors than the cells before selection, indicating the efficient amplification of the transduced cells over the non-transduced cells. Furthermore, Ba/HgIGFP+Lg and Ba/HE+LgIGFP cells selected without any factors also expressed high amounts of chimeric receptors comparable to the HEL-selected cells, further confirming the background growth signal from these chimeric receptor combinations. These results demonstrate the preferential amplification of the transfectants expressing chimeric receptor combinations by direct HEL selection without antibiotic selection.

### HEL selection induced selective expansion of EGFP-positive cells

To examine whether HEL selection induced selective expansion of the cells with transgene expression as well as chimeric receptor expression, flow cytometric analysis was performed to determine the EGFP-positive cell population before and after selection. On day 3 or 4 after infection, all the transfectants showed an EGFP-positive cell ratio of <5% (Figs 3–5). After IL-3 selection, the selected cells exhibited

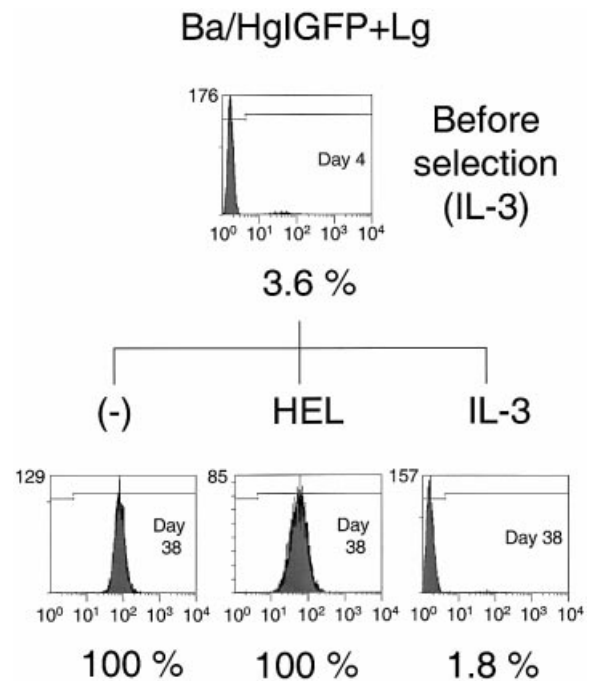


**Figure 2.** Antigen-mediated expansion of the transfectants with chimeric receptor expression. The expression level of the chimeric receptor was compared before (bef.) and after (aft.) selection. Selection was performed in the culture medium containing either no factors (-), 1 µg/ml HEL or 2 ng/ml IL-3. pMX means Ba/F3 cells transfected with pMX vector without any inserts (named Ba/pMX) as a negative control. (A) Hg+Lg combinations, (B) HE+Lg combinations and (C) Hg+LE combinations were analyzed with anti-gp130 and anti-EpoR C-terminus antibodies. In each lane, cell lysate from  $1.1 \times 10^5$  cells was loaded. The actual dates for the harvest were as follows. In (A), day 6 for HgIGFP, Lg and HgIGFP+Lg bef.; day 39 for aft. (-) and HEL; day 36 for IL-3. In (B), day 7 for LgIGFP, HE and HE+LgIGFP bef.; day 67 for aft. (-); day 37 for HEL; day 56 for IL-3. In (C), day 6 for HgIGFP; day 7 for LEIGFP and Hg+LEIGFP bef.; day 40 for aft. HEL; day 56 for IL-3; day 6 for HgIGFP+LE bef.; day 39 for aft. HEL; day 36 for IL-3.

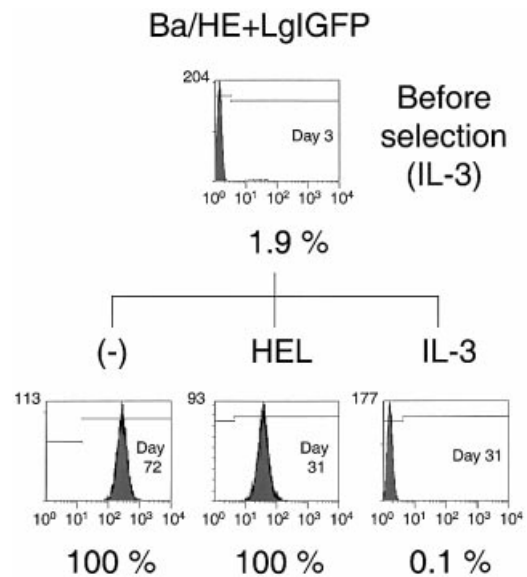
similar EGFP-positive ratios to the cells before selection. On the other hand, the HEL-selected cells were 100% EGFP-positive for all amplifier-courier combinations. Furthermore, Ba/HgIGFP+Lg and Ba/HE+LgIGFP cells cultured without any factors also exhibited an EGFP-positive ratio of 100% (Figs 3 and 4). The EGFP-positive ratio was closely related to the expression level of the chimeric receptors, indicating efficient coupling of the two cistrons located upstream and downstream of the IRES sequence. These results clearly demonstrate that the chimeric receptor-derived growth signal induced highly efficient amplification of EGFP-positive cells.

**HEL-dependent cell growth of the transfectants**

To confirm the characteristics of HEL-dependent cell growth of the transfectants after HEL selection, a cell proliferation assay of the transfectants was performed. Cells were washed and cultured in various concentrations of HEL. During the

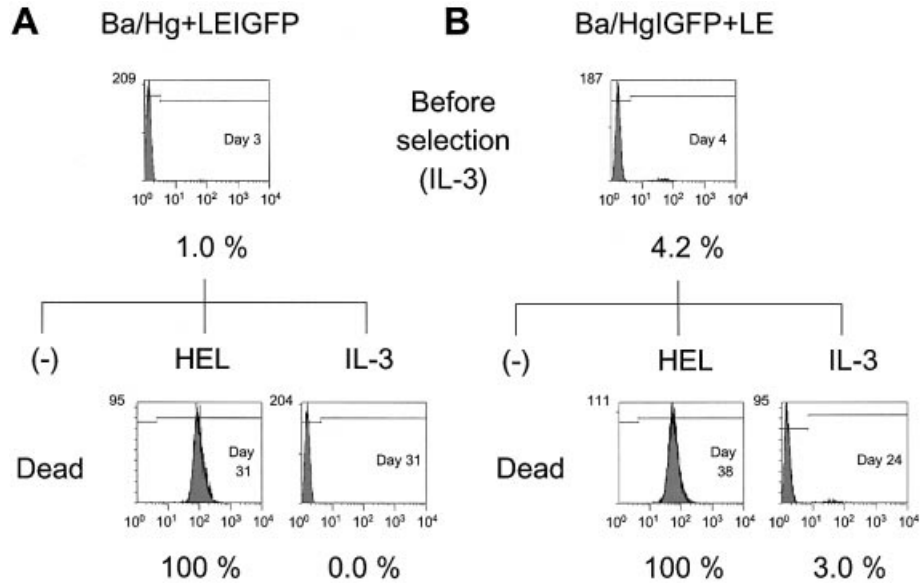


**Figure 3.** Selective expansion of EGFP-positive cells with Hg+Lg combinations. Cells ( $10^4$ ) were analyzed with flow cytometry, and cell number was plotted against log green fluorescence intensity. EGFP-negative and EGFP-positive regions were determined by taking Ba/pMX cells as a negative control. The day from retroviral infection and the EGFP-positive ratio are indicated.

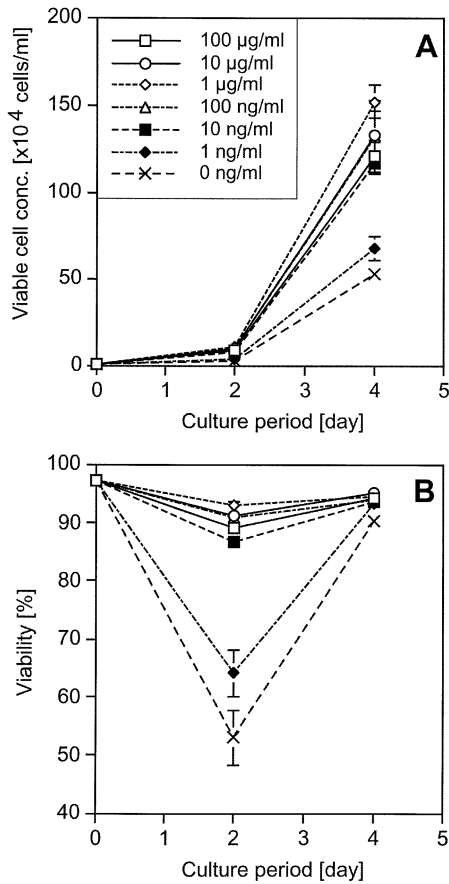


**Figure 4.** Selective expansion of EGFP-positive cells with HE+Lg combinations. Cell number is plotted against log green fluorescence intensity. The day from retroviral infection and the EGFP-positive ratio are indicated.

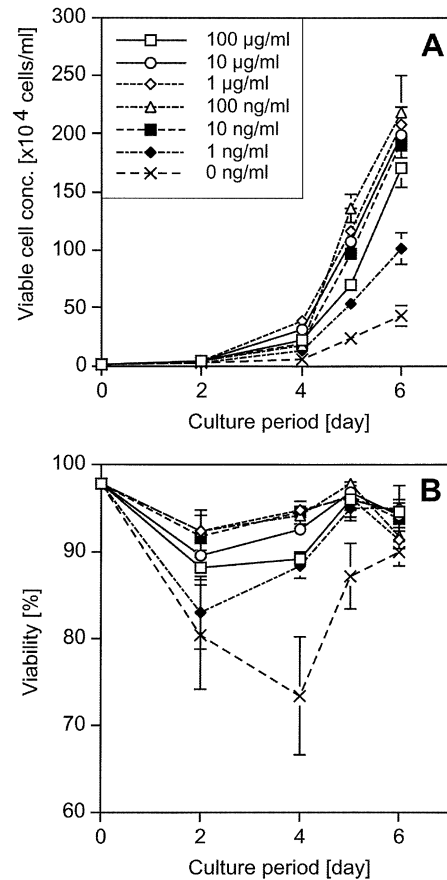
culture period, cell density and viability were measured. Ba/HgIGFP+Lg cells showed HEL-dependent cell growth with a lower limit of 10 ng/ml, although considerable background growth without HEL was observed (Fig. 6). In the case of Ba/HE+LgIGFP cells, the lower limit of HEL-dependent cell growth was 1 ng/ml, which was most sensitive of all the amplifier-courier combinations (Fig. 7). However,



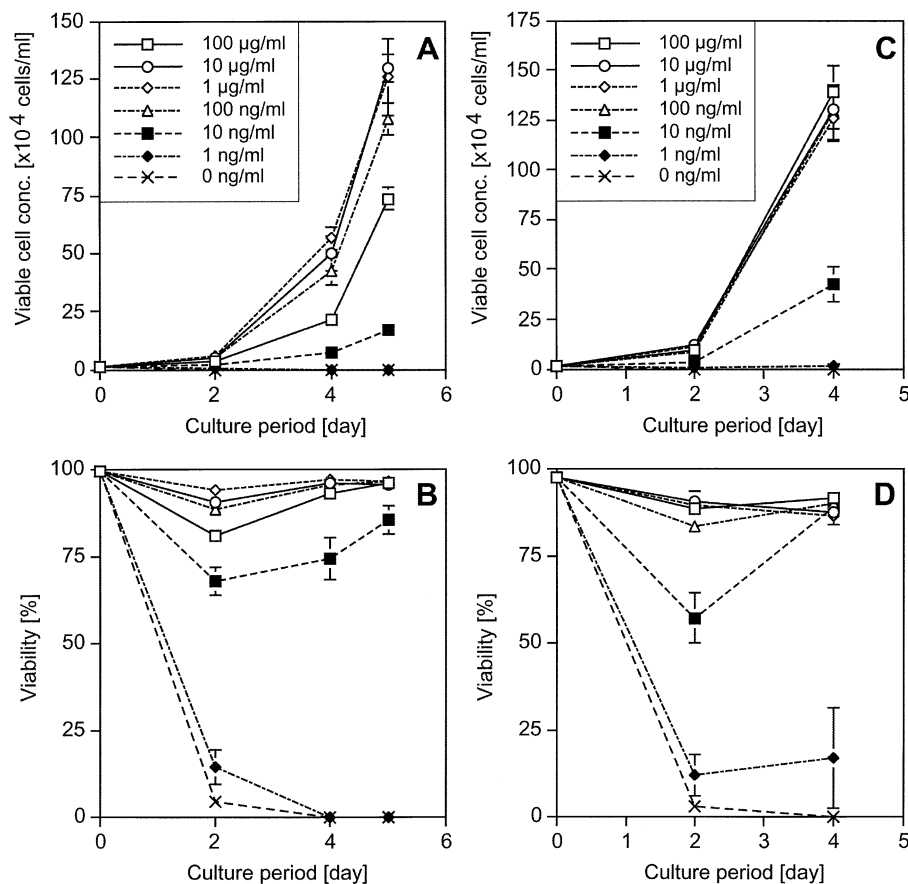
**Figure 5.** Selective expansion of EGFP-positive cells with Hg+LE combinations. Cell number is plotted against log green fluorescence intensity. The day from retroviral infection and the EGFP-positive ratio are indicated. (A) Ba/Hg+LEIGFP cells. (B) Ba/HgIGFP+LE cells.



**Figure 6.** Growth and viability curves of Ba/HgIGFP+Lg cells. Cells ( $10^4$  cells/ml) were inoculated into 24-well plates at day 0. Viable cell concentration and viability of triplicates are plotted with average and 1 SD. (A) HEL-dependent growth of Ba/HgIGFP+Lg cells. (B) Viability of the same cells, where the keys are as in (A).



**Figure 7.** Growth and viability curves of Ba/HE+LgIGFP cells. Cells ( $10^4$  cells/ml) were inoculated into 24-well plates at day 0. Viable cell concentration and viability of triplicates are plotted with average and 1 SD. (A) HEL-dependent growth of Ba/HE+LgIGFP cells. (B) Viability of the same cells, where the keys are as in (A).



**Figure 8.** Growth and viability curves of the transfectants with Hg+LE combinations. Cells ( $10^4$  cells/ml) were inoculated into 24-well plates at day 0. Viable cell concentration and viability of triplicates are plotted with average and 1 SD. (A) HEL-dependent growth of Ba/Hg+LEIGFP cells. (B) Viability of the same cells, where the keys are as in (A). (C) HEL-dependent growth of Ba/HgIGFP+LE cells. (D) Viability of the same cells.

background cell growth without HEL, which was less efficient than that induced by Hg+Lg combinations, was also observed. On the other hand, Ba/HgIGFP+LE and Ba/Hg+LEIGFP showed strict HEL-dependent cell growth without background growth (Fig. 8). The lower limit of HEL required for growth was 10 ng/ml, although 1 ng/ml HEL showed some anti-apoptotic effect. From these analyses, the Hg+LE combination was found to be the best one as an inducible selective amplifier in Ba/F3 cells.

#### Comparison of the selection efficiency with antibiotic selection

To investigate the selection efficiency of our Antigen Mediated Genetically modified cell Amplification (AMEGA) system, a cloning experiment was performed. Ba/HE+LgIGFP and Ba/Hg+LEIGFP cells before selection were inoculated into two 96-well plates at a cell concentration of  $10^3$  cells/well. From the EGFP-positive cell ratio before selection, given that the gene transfer efficiencies of both courier and amplifier vectors are the same, the populations of co-transfectants are calculated to be 0.036% (= 1.9%) and 0.01% (= 1.0%) for Ba/HE+LgIGFP and Ba/Hg+LEIGFP cells, respectively. As shown in Table 1, Ba/HE+LgIGFP and Ba/Hg+LEIGFP showed selection efficiencies of up to 30 and 26%. Although the values were not more than that with antibiotic selection

**Table 1.** Comparison of the selection efficiency

Cells	No. positive wells	Expected clone no.	Selection efficiency (%)
Ba/HE+LgIGFP	21	69	30
Ba/Hg+LEIGFP	5	19	26
Ba/neoIGFP	30	67	45

The cells were inoculated into two 96-well plates with an initial cell density of 1 cell/well (for Ba/neoIGFP) or  $10^3$  cells/well (for the others). Number of positive wells out of 192 wells for cell growth is shown. Expected clone number was calculated from the EGFP-positive cell ratio before selection on the assumption that transduction efficiency of an amplifier vector was the same as that of a cognate courier vector in each combination.

of 45%, the data suggests that our AMEGA system could be almost as efficient as antibiotic selection as a selection method.

#### DISCUSSION

In this study, we have described an AMEGA system without any antibiotic selection steps. Antibiotic selection induces some deleterious effects such as growth inhibition (2,3), mutations in the antibiotic resistance gene and cell membrane permeability (19,20) and morphological changes (21). In

contrast, in our system only one-step antigen selection resulted in 100% transgene-positive cells without any deleterious effects, indicating the utility of our method over conventional antibiotic selection.

Compared with antibiotic selection, the selection efficiency of our AMEGA system was almost comparable. In addition, the AMEGA system could be potentially used for *in vivo* selection of transduced cells, which could not be attained by cytotoxic drug selection. We believe that this is a particular advantage of the AMEGA system, and that the selection efficiency of our system is sufficiently high for practical use.

Whereas Ba/HgIGFP and Ba/LgIGFP cells did not exhibit background growth, Hg+Lg and HE+Lg combinations elicited some background growth without HEL, indicating preformed Hg-Lg or HE-Lg heterodimers in the transfectant expressing Hg+Lg or HE+Lg combinations, respectively. Although the background growth of the transfectant expressing Hg+Lg was observed in previous studies (11,12), the background growth of Ba/HE+LgIGFP was an unexpected finding, since the Hg+LE combination exhibited strict HEL-dependent cell growth. The distinct characteristics of Hg+LE and HE+Lg imply that the small conformational difference has an effect on signal transduction in the artificially induced EpoR-gp130 heterodimer. Recent X-ray crystallographic analyses revealed the importance of the conformation of the extracellular domain in EpoR signaling (8,9,22–24). In addition, recent studies using mutagenesis or chimeric receptors demonstrated the contribution of association between receptor transmembrane domains in EpoR signaling (25,26). In the model proposed in the study (26), although EpoR takes two dimeric conformations with association in the extracellular domains or the transmembrane domains in the absence of Epo, the latter conformation is stabilized by Epo binding, thereby triggering signal transduction. Therefore, the different characteristics of Hg+LE and HE+Lg might imply that the stabilization effect of the transmembrane domain was changed by the extracellular construct of the chimera. The alteration to the mutated transmembrane domain with weaker association might realize more strictly regulated antigen-dependent cell amplification (25–28).

Since antibiotic selection inhibits cell growth, it may take more time to select and expand the transfectant. The long selection period is problematic for the selection of primary culture cells, especially for the ones in which transduction efficiency is low, since the cells might be unexpectedly differentiated during the selection. In contrast, our method has the advantage that the cell growth could be rather stimulated. Stem cells, due to their pluripotency, are promising targets for gene therapy to correct various diseases (29–31). Since hematopoietic stem cells were reported to be expanded with gp130 and EpoR signaling (32,33), Hg+LE combinations might be applied to the *ex vivo* or *in vivo* amplification of genetically modified hematopoietic stem cells. Generalization of this approach including the use of other functional antigen–antibody pairs and alternative intracellular domains will provide wide applications of our selection system.

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